

## CLAIMS

1. A method for the continuous, semi-continuous or discontinuous treatment of a substrate (24), in which said substrate (24) installed in a bioreactor vessel (1) is subjected to the action of a culture of living cells C1 making it possible to carry out a reaction R1 on said substrate (24) and in which the medium is inoculated periodically using living cells C2 improving said reaction, said living cells C2 originating from a selection carried out by an automatic device for selecting living cells (2), from a population of dynamic living cells and said automatic device (2) for selecting living cells being supplied either by a different substrate or by the same substrate (24) as the bioreactor vessel (1) and being inoculated at the outset by the living cells C1 present in the tank of the bioreactor vessel (1), and in which living cells are removed from the tank of the bioreactor vessel in order to be transferred into the automatic selection device (2).

2. A method according to claim 1, characterized in that the automatic device (2) for selecting dynamic living cells comprises:

- two or more vessels (20, 21) making it possible to receive and maintain cultures of living cells in suspension,
- a set of means making it possible to separately supply these vessels with sterilizing (25), cleaning or neutralizing liquids,
- a set of means making it possible to supply these vessels with gas (23),
- a set of means making it possible to supply these vessels with substrate (24),
- a set of means (28-31) making it possible to transfer the content of one vessel (20) into the other (21) and vice-versa,
- a set of means making it possible to evacuate all or part of the content of these vessels to another device such as a bioreactor vessel (1),
- a set of means making it possible to evacuate all or part of the content of these vessels (20, 21) to a refuse bin.

3. A method according to claim 1 or 2, characterized in that the automatic device for selecting dynamic living cells comprises in particular

- (a) at least one first and at least one second culture vessel (20, 21) intended to receive a culture (22),
- (b) a source of gas (23);
- (c) a source of medium (substrate) (24);
- 5 (d) a source (25) for a sterilizing agent; and
- (e) a system of pipes comprising means for connecting either one of the two culture vessels (20, 21) to the source of medium (24) such as valves as well as for connecting the two culture vessels (20, 21) to each other and for connecting either of the other culture vessels (20 or 21) to the source
- 10 (25) of the sterilizing agent.

4. A method according to one of claims 1 to 3, characterized in that the living cells C2 originate from the selection carried out from a population of dynamic living cells exclusively in suspension.

- 15 5. A method according to one of claims 1 to 4, characterized in that the bioreactor vessel (1) is an aeration tank of a treatment plant, the methanization tank of an anaerobic biological treatment unit, a lagoon, a reservoir, a container for example from 0.5 litre to 100 m<sup>3</sup> or a fermenter.

- 20 6. A method according to one of claims 1 to 5, characterized in that the living cells C2 used for improving the bioconversion reaction can in particular be produced by implementation of a method comprising the following stages:
  - (a) making available a culture (22) in at least one first culture vessel (20);
  - (b) continuous supplying of the culture (22) in the first culture vessel (20) with gas from a source of gas (23) and regular replenishment with liquids from a source of substrate (24),
  - 25 (c) transfer of the culture (22) from the first culture vessel (20) by connecting pipes (28-31) into at least one second culture vessel (21) by means of an appropriate pipe circuit,
  - (d) connection of the first culture vessel (20) to a source (25) for a sterilizing agent, in order to sterilize the first culture vessel (20),
  - 30 (e) removal of the sterilizing agent from the first culture vessel (20),
  - (f) continuous supplying of the culture (22) in the second culture vessel (21) with gas from the source of gas (23) and regular replenishment with liquids from the source of medium (24),

- (g) return of the culture (22) from the second culture vessel (21) via the connecting pipes (28-31) into the first culture vessel (20) by means of an appropriate pipe circuit,
- (h) connection of the second culture vessel (21) to the source (25) for the sterilizing agent, in order to sterilize the second culture vessel (21); and
- (i) removal of the sterilizing agent from the second culture vessel (21).

7. A method according to one of claims 1 to 6, characterized in that the substrate (24) is

- a medium containing a compound the metabolic conversion of which is envisaged, for example, a water of industrial origin, water of municipal origin such as for example domestic sewage, an accidental pollutant of the environment such as for example the presence in the sea of a slick of hydrocarbons or other chemical products, chemical effluents spread on the ground, soil polluted with heavy metals or dioxin or
- a compound the metabolic conversion of which is envisaged, for example, glucose, ethanol or oxalic acid, or
- a volatile organochlorinated compound, an organochlorinated pesticide, a halogenated polycyclic aromatic hydrocarbon or a solvent.

8. A method according to one of claims 1 to 7, characterized in that the living cell comprises one or more bacterial species, animal or plant cells or cells of algae, yeasts or fungi.

9. A method according to one of claims 1 to 8, characterized in that the periodic inoculation originating from the automatic device (2) for selecting living cells is for example carried out at least once a week.

10. A device for culturing living cells comprising:

- A: a device for selecting living-cells C2 improving a reaction R1 of bioconversion of a substrate (24), said living cells C2 being variants deriving from living cells C1, and said living cells C2 producing an improved reaction relative to that produced by the living cells C1,
- B: a bioreactor vessel (1),
- C: a system of pipes (5) comprising means for carrying out transfers from the selection device (2) to the bioreactor vessel (1) and a system of pipes (6) comprising means for carrying out transfers from the bioreactor vessel (1) to the selection device (2),

- D: optionally a pipe (15) comprising means for connecting the bioreactor vessel (1) to a solid-liquid separation device such as a settling tank (16),
- E: optionally a pipe for evacuation of the liquid (water for example) treated,
- F: optionally a temperature regulation device.

5        11. A device for culturing living cells according to claim 10, characterized in that the selection device (2) comprises

- two or more vessels (20, 21) making it possible to receive and maintain cultures of living cells in suspension,
- a set of means making it possible to supply these vessels with substrate
- 10        (24),
- a set of means (28-31) making it possible to transfer the content of one vessel (20) into the other (21) and vice-versa,
- a set of means making it possible to evacuate all or part of the content of these vessels to another device such as a bioreactor vessel (1),
- 15        - a set of means making it possible to evacuate all or part of the content of these vessels (20, 21) to a refuse bin.

12. A device for culturing living cells by coupling with an automatic device for selecting living cells according to claim 11 comprising:

- A: a device for selecting living cells (2) comprising
- 20        (a) at least one first and at least one second culture vessel (20, 21) intended to receive a culture (22),
- (b) a source of gas (23),
- (c) a source of medium (24),
- (d) a source (25) for a sterilizing agent; and
- 25        (e) a system of pipes comprising means for connecting either one of the two culture vessels (20 or 21) to the source of medium (24) such as valves as well as connecting the two culture vessels (20, 21) to each other and for connecting either one of the other culture vessels (20 or 21) to the source (25) of the sterilizing agent.
- 30        - B: a bioreactor vessel (1)
- C: a system of pipes (5, 6) comprising means for connecting the selection device to the bioreactor vessel (1),
- D: optionally a pipe (15) comprising means for connecting the bioreactor vessel (1) to a solid-liquid separation device such as a settling tank (16),

- E: optionally a pipe (7) for evacuation of the liquid (water for example) treated.
- F: optionally a temperature regulation device.